Change in Plasma Renin Activity by Cold Storage of Plasma in Normal Subjects and Patients with Essential Hypertension and Primary Aldosteronism

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SAITO, K., ABE, K., ITO, T., IROKAWA, N., OTSUKA, Y., KUSAKA, T., YASUJIMA, M., IMAI, Y., SAKURAI, Y., CHIBA, S., RITSU, K., SATO, M., HARUYAMA, T., OMATA, K. and YOSHINAGA, K. Change in Plasma Renin Activity by Cold Storage of Plasma in Normal Subjects and Patients with Essential Hypertension and Primary Aldosteronism. Tohoku J. exp. Med., 1979, 128 (1), 13-18 — The time courses of change in renin activity after cold storage of human plasma at -5°C and pH 7.4 were examined in 5 normal subjects, 6 patients with essential hypertension and one female patient with primary aldosteronism before and after extirpation of the adrenal tumor. In the 5 normal subjects and 6 essential hypertensives, the gradual increase in plasma renin activity was observed until 10 days of cold storage. The same result was obtained in the case of primary aldosteronism. However, there was no increase in renin activity despite of cold storage for 10 days in plasma which was sampled from this patient 45 days after operation. These data indicate that a period of 4 days for cryoactivation of human plasma renin as has been reported by Sealey et al. is not sufficient to accomplish activation of renin by cold storage.

Increase in renin activity of human plasma after cold storage was first reported by Osmond et al. (1973). Sealey and Laragh (1975) have suggested that human plasma contains an inactive form of renin which is converted to the active form by cryoactivation, and they called this form of renin "prorenin". Sealey et al. (1976) have also showed that cryoactivation was accomplished by incubating plasma at -5°C, pH 7.4, for 4 days.

The purpose of the present study was to investigate the time course of cryoactivation of human plasma renin in normal subjects, patients with essential hypertension, and a patient with primary aldosteronism before and after surgical treatment.

MATERIALS AND METHODS

Five normal subjects (4 men and one woman aged 18–30 with a mean of 27 years), 6 patients with essential hypertension (2 men and 4 women aged 20–64 with a
mean of 41 years) and one female patient with primary aldosteronism aged 33 were studied in this experiment. None of the normal subjects had a history of hypertension and all had a casual blood pressure of 130/80 mm Hg or less. The clinical diagnosis of essential hypertension was made by physical and laboratory examinations, renal angiography, and determinations of plasma aldosterone and urinary 17-OHCS and catecholamines. They had a diastolic blood pressure of 90 mm Hg or above on repeated measurements and no cardiovascular complications. We confirmed the diagnosis of primary aldosteronism later by operation. All antihypertensive medications were discontinued 2 weeks prior to sampling of blood in patients with essential hypertension while 100 mg of spironolactone and 72 mEq of potassium was daily administered in the patient with primary aldosteronism. All subjects were allowed to take unrestricted sodium diet containing approximately 200 mEq of sodium daily. All patients were studied in Tohoku University Hospital.

The time course of cryoactivation of plasma renin activity was examined as follows. Plasma renin activity was measured before and after 1, 2, 3, 4, 10, 30 and 60 days of cold storage at -5°C and pH 7.4 in plasma from normal subjects and patients with essential hypertension. In primary aldosteronism, cryoactivation was examined in plasma which was sampled before and 8 hr, 1, 3, 4, 5 and 45 days after operation. The method of Sealey et al. was used for cryoactivation of plasma. For cold storage of the plasma, all samples were stocked in polyethylene tube and placed in a freezer set at -5°C.

Plasma renin activity was measured using radioimmunoassay of generated angiotensin I by incubation of plasma at 37°C, pH 5.5 for 6 hr with disodium ethylenediamine tetra-acetic acid (EDTA) and di-isopropyl fluorophosphate (DFP) as previously described (Abe et al. 1972). One ml of plasma was used for radioimmunoassay in determining plasma renin activity without cold storage at -5°C while 0.5 ml of that after cold storage. After 1, 2, 3, 4, 10, 20 and 60 days of cold storage, amount of angiotensin I was measured in plasma without incubation at 37°C and pH 5.5 in order to examine the possibility that generation of angiotensin I occurs during cold storage of plasma and this was negligible because all the samples showed no detectable amount. Samples stored frozen at -20°C for 30 and 60 days exhibited no increase in plasma renin activity.

Plasma aldosterone concentration was determined by the radioimmunoassay using commercial kits (CIS, Midorijuji) (normal 0.5–12.6 ng/100 ml).

**RESULTS**

Fig. 1 demonstrates the time courses of cryoactivation of renin activity in plasma from 5 normal subjects. In all samples a gradual increase in plasma renin activity was found until 10 days of cold storage. Average plasma renin activity before and after 1, 2, 3, 4, 10, 20 and 60 days of cold storage was 6.5±2.1 (mean ±s.e.) ng/ml, 7.4±2.4 ng/ml, 11.8±4.1 ng/ml, 13.4±4.6 ng/ml, 15.9±5.0 ng/ml, 36.5±10.4 ng/ml, 39.6±12.1 ng/ml and 45.0±10.0 ng/ml, respectively. Average plasma renin activity after 10 days of cold storage was significantly higher than that without cold storage (p<0.05), while that after 4 days of cold storage was not. No further significant increase was found after 30 days of cold storage in any samples except in Case 3, in which plasma renin activity increased from 45 ng/ml after 10 days of cold storage to 70 ng/ml after 30 days of cold storage.

Fig. 2 shows the results in plasma samples from 6 essential hypertensives. In all samples, a gradual increase in plasma renin activity was found until 10 days of cold storage. Average plasma renin activity before and after 1, 2, 3, 4, 10, 20 and 60 days of cold storage was 6.2±3.8 ng/ml, 9.4±2.5 ng/ml, 8.6±3.1 ng/ml, 11.3±3.7 ng/ml, 11.8±3.2 ng/ml, 19.8±6.3 ng/ml, 27.7±7.6 ng/ml and 31.9±6.0 ng/ml.
Fig. 1. Increase in plasma renin activity in 5 normal subjects. ○, Case 1; ●, Case 2; △, Case 3; ▲, Case 4; ▼, Case 5.

Fig. 2. Increase in plasma renin activity in 6 patients with essential hypertension. ○, Case 6; ●, Case 7; △, Case 8; ▲, Case 9; ▼, Case 10; ▼, Case 11.

respectively. Average plasma renin activity after 10 days of cold storage was significantly higher than that without cold storage (p<0.01). Further increase in plasma renin activity was found in 3 samples after 30 days and 60 days of cold storage.
Fig. 3 illustrates the time courses of cryoactivation of renin activity in samples from a female patient with primary aldosteronism before and after surgical treatment. Plasma renin activity before cold storage all showed trace throughout the observed period. A significant increase in plasma renin activity was found after 4 days of cold storage (9.8±4.3 ng/ml), compared with that without cold storage (all trace) (p<0.02) with the exception that there was no increase in renin activity in plasma sampled 45 days after operation. Further increase in plasma renin activity was observed after 10 days of cold storage (13.0±2.4) with the same exception as found after 4 days of cold storage (p<0.005). There was no increase in plasma renin activity during cold storage of plasma sampled from this patient 45 days after operation when this patient was still hypertensive without medication. However, plasma aldosterone concentration was normal (4.5 ng/100 ml).

There was no significant correlation between changes in plasma renin activity after 10 days of cold storage and plasma aldosterone concentration in the samples from normal subjects and patients with essential hypertension and primary aldosteronism before and after surgical treatment. There was no significant correlation between plasma renin activity without cold storage and changes in plasma renin activity after 10 days of cold storage in normal subjects and essential hypertensives.
DISCUSSION

Osmond et al. (1973) first reported an increase in plasma renin activity of up to 300% after cold storage of human plasma for 3 days at 4°C and an additional increase by longer storage (9–31 days) in normal males. They suggested that the increase in plasma renin activity was due to the activation of renin or to the degradation of renin inhibitor by cold storage of plasma. Recently Sealey and Laragh (1975) have suggested that cryoactivation of plasma renin is due to neither activation of renin activators nor degradation of renin inhibitors. Although our data do not show whether cryoactivation of plasma renin activity is due to activation of inactive renin, it is clear that there is an increase in plasma renin activity after cold storage of human plasma at -5°C and pH 7.4. This is in accordance with the results of Sealey and Laragh. In our experiment, however, there was a further increase in plasma renin activity after cold storage of plasma beyond days in contrast to the study of Sealey and Laragh, in which they showed cryoactivation is accomplished by placing plasma in a shaker water bath at -5°C for 4 days. One of the possibilities for this discrepancy might be that we placed samples in a freezer in preincubation period instead of shaker bath. Though the reason for this discrepancy is not clear, our data show that a period of 4 days of incubation of plasma at -5°C is not enough to accomplish cryoactivation of renin activity of human plasma. We suppose that a period of at least 10 days of preincubation is necessary for accomplishing cryoactivation. These results indicate that it is difficult to assess an importance of prorenin by cryoactivation of plasma for only 4 days. In the present study, there was no increase in plasma renin activity in samples stored frozen at -20°C for 30 and 60 days. These results are in accordance with the study of Sealey et al. (1976) in which samples stored frozen at -40°C for over a year have exhibited no activation of prorenin.

As illustrated in Fig. 3, there was no increase in renin activity in plasma sampled from the patient with primary aldosteronism 45 days after operation. At that time plasma aldosterone concentration was normal. No report has been made about plasma renin activities after cold storage in primary aldosteronism, needless to say the comparison of those before and after surgical treatment. Further study will be necessary to explain this result.

In conclusion, we have suggested that a period of 4 days of incubation at -5°C and pH 7.4 is not sufficient to accomplish cryoactivation of renin.

References
